

Amendments to the Specification

Please replace paragraph [0006] with the following amended paragraph:

[0006] It is well recognized that reversible phosphorylation of proteins controls many cellular processes in plants and animals. The phosphorylation status of proteins is regulated by the opposing activities of protein kinases and protein phosphatases. Phosphorylation of eukaryotic proteins occurs predominantly on serine and threonine residues, and to a lesser extent, on tyrosine residues. In animals, protein ~~pheshporylation~~ phosphorylation plays well-known roles in diverse cellular processes such as glycogen metabolism, cell cycle control, and signal transduction (Smith, R.D. and Walker, J.C., 1996, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:101-125).

Please replace paragraph [0012] with the following amended paragraph:

[0012] The invention provides in some embodiments that the PHSRP and coding nucleic acid are ~~that~~ those found in members of the genus *Physcomitrella*. In another preferred embodiment, the nucleic acid and protein are from a *Physcomitrella patens*. The invention provides that the environmental stress can be salinity, drought, temperature, metal, chemical, pathogenic and oxidative stresses, or combinations thereof. In preferred embodiments, the environmental stress can be drought or cold temperature.

Please replace paragraph [0034] with the following amended paragraph:

[0034] The invention further describes isolated PHSRPs. In one preferred embodiment, the PHSRPs are isolated from the plant genus *Physcomitrella*. In another preferred embodiment, the PHSRPs are from a *Physcomitrella patens* (*P. patens*) plant. The present invention describes for the first time the predicted *P. patens* proteins PP2A-2 (SEQ ID NO:11), PP2A-3 (SEQ ID NO:12) and PP2A-4 (SEQ ID NO:13) that are homologous to protein phosphatase 2A. Other novel predicted proteins described herein are PP2C-1 (SEQ ID NO:14) and PP2C-2 (SEQ ID NO:15) that are homologous to protein phosphatase 2C. Accordingly, in a preferred embodiment, the PHSRP is a protein ~~pheshpatase~~ phosphatase 2A protein, a protein phosphatase 2C protein or a homolog or an ortholog thereof. In a further preferred embodiment, the PHSRP is selected from the

group consisting of PP2A-2 (SEQ ID NO:11), PP2A-3 (SEQ ID NO:12), PP2A-4 (SEQ ID NO:13), PP2C-1 (SEQ ID NO:14), PP2C-2 (SEQ ID NO:15) and homologs and orthologs thereof. Homologs and orthologs of amino acid sequences are defined below.

Please replace paragraph [0100] with the following amended paragraph:

[0100] For such plant transformation, binary vectors such as pBinAR can be used (Höfgen and Willmitzer, 1990 Plant Science 66:221-230). Construction of the binary vectors can be performed by ligation of the cDNA in sense or antisense orientation into the T-DNA. 5-prime Five-prime to the eDNA cDNA, a plant promoter activates transcription of the cDNA. A polyadenylation sequence is located 3-prime to the cDNA. Tissue-specific expression can be achieved by using a tissue specific promoter. For example, seed-specific expression can be achieved by cloning the napin or LeB4 or USP promoter 5-prime to the cDNA. Also, any other seed specific promoter element can be used. For constitutive expression within the whole plant, the CaMV 35S promoter can be used. The expressed protein can be targeted to a cellular compartment using a signal peptide, for example for plastids, mitochondria or endoplasmic reticulum (Kermode, 1996 Crit. Rev. Plant Sci. 4 (15):285-423). The signal peptide is cloned 5-prime in frame to the cDNA to archive achieve subcellular localization of the fusion protein. Additionally, promoters that are responsive to abiotic stresses can be used with, such as the *Arabidopsis* promoter RD29A, the nucleic acid sequences disclosed herein. One skilled in the art will recognize that the promoter used should be operatively linked to the nucleic acid such that the promoter causes transcription of the nucleic acid which results in the synthesis of a mRNA which encodes a polypeptide. Alternatively, the RNA can be an antisense RNA for use in affecting subsequent expression of the same or another gene or genes.

Please replace paragraph [0101] with the following amended paragraph:

[0101] Alternate methods of transfection include the direct transfer of DNA into developing flowers via electroporation or *Agrobacterium* mediated gene transfer. *Agrobacterium* mediated plant transformation can be performed using for example the

GV3101(pMP90) (Koncz and Schell, 1986 Mol. Gen. Genet. 204:383-396) or LBA4404 (Clontech) *Agrobacterium tumefaciens* strain. Transformation can be performed by standard transformation and regeneration techniques (Deblaere et al., 1994 Nucl. Acids. Res. 13:4777-4788; Gelvin, Stanton B. and Schilperoort, Robert A, Plant Molecular Biology Manual, 2nd Ed. – Dordrecht : Kluwer Academic Publ., 1995. – in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R.; Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993. – 360 S.,ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989 Plant cell Report 8:238-242; De Block et al., 1989 Plant Physiol. 91:694-701). Use of antibiotica antibiotics for *Agrobacterium* and plant selection depends on the binary vector and the *Agrobacterium* strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. *Agrobacterium* mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994 Plant Cell Report 13:282-285. Additionally, transformation of soybean can be performed using using, for example example, a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent Application No. 0397 687, U.S. Patent No. 5,376,543 or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbide fiber technique. (See, for example, Freeling and Walbot "The maize handbook Maize Handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387 and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

Please replace paragraph [0132] with the following amended paragraph:

[0132] Culturing was carried out in a climatic chamber at an air temperature of 25°C and light intensity of 55 micromols⁻¹m⁻² micromol s⁻¹m⁻² (white light; Philips TL 65W/25 fluorescent tube) and a light/dark change of 16/8 hours. The moss was either modified in liquid culture using Knop medium according to Reski and Abel (1985, Planta 165:354-358) or cultured on Knop solid medium using 1% oxoid agar (Unipath, Basingstoke,

England). The protonemas used for RNA and DNA isolation were cultured in aerated liquid cultures. The protonemas were comminuted every 9 days and transferred to fresh culture medium.

Please replace paragraph [0172] with the following amended paragraph:

[0172] Seeds of soybean ~~were~~ are surface sterilized with 70% ethanol for 4 minutes at room temperature with continuous shaking, followed by 20% (v/v) Clorox supplemented with 0.05% (v/v) Tween for 20 minutes with continuous shaking. Then, the seeds ~~were~~ are rinsed 4 times with distilled water and placed on moistened sterile filter paper in a Petri dish at room temperature for 6 to 39 hours. The seed coats ~~were~~ are peeled off, and cotyledons are detached from the embryo axis. The embryo axis ~~was~~ is examined to make sure that the meristematic region is not damaged. The excised embryo axes ~~were~~ are collected in a half-open sterile Petri dish and air-dried to a moisture content less than 20% (fresh weight) in a sealed Petri dish until further use.

Please replace paragraph [0173] with the following amended paragraph:

[0173] *Agrobacterium tumefaciens* culture ~~was~~ is prepared from a single colony in LB solid medium plus appropriate antibiotics (e.g. 100 mg/l streptomycin, 50 mg/l kanamycin) followed by growth of the single colony in liquid LB medium to an optical density at 600 nm of 0.8. Then, the bacteria culture ~~was~~ is pelleted at 7000 rpm for 7 minutes at room temperature, and resuspended in MS (Murashige and Skoog, 1962) medium supplemented with 100 μ M acetosyringone. ~~Bacteria~~ Bacterial cultures ~~were~~ are incubated in this pre-induction medium for 2 hours at room temperature before use. The ~~axis~~ axes of soybean zygotic seed embryos at approximately 15% moisture content ~~were~~ are imbibed for 2 hours at room temperature with the pre-induced *Agrobacterium* suspension culture. The embryos are removed from the imbibition culture and ~~were~~ are transferred to Petri dishes containing solid MS medium supplemented with 2% sucrose and incubated for 2 days, in the dark at room temperature. Alternatively, the embryos ~~were~~ are placed on top of moistened (liquid MS medium) sterile filter paper in a Petri dish and incubated under the same conditions described above. After this period, the

embryos ~~were~~ are transferred to either solid or liquid MS medium supplemented with 500 mg/L carbenicillin or 300mg/L cefotaxime to kill the agrobacteria. The liquid medium was ~~is~~ used to moisten the sterile filter paper. The embryos ~~were~~ are incubated during 4 weeks at 25°C, under 150 $\mu\text{mol m}^{-2}\text{sec}^{-1}$ and 12 hours photoperiod. Once the seedlings produced produce roots, they ~~were~~ are transferred to sterile metromix soil. The medium of the *in vitro* plants was ~~is~~ washed off before transferring the plants to soil. The plants ~~were~~ are kept under a plastic cover for 1 week to favor the acclimatization process. Then the plants ~~were~~ are transferred to a growth room where they ~~were~~ are incubated at 25°C, under 150 $\mu\text{mol m}^{-2}\text{sec}^{-1}$ light intensity and 12 hours photoperiod for about 80 days.

Please replace paragraph [0174] with the following amended paragraph:

[0174] The transgenic plants ~~were~~ are then screened for their improved drought, salt and/or cold tolerance according to the screening method described in Example 7 demonstrating to demonstrate that transgene expression confers stress tolerance.